Introduction to and Principles of Confinement

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ANIMAL AND PLANT HEALTH INSPECTION SERVICE

Biotechnology Regulatory Services

September 13, 2004

Title 7 Code of Federal Regulations Part 340.4 (std. permit conditions)

"The regulated article shall be":

- kept separate from other organisms (except as allowed in the permit)
- maintained only in the areas and premises specified in the permit
- subject to the application of measures determined to be necessary to prevent the accidental or unauthorized release…

1989 National Research Council Report Field Testing Genetically Engineered Plants: Framework for Decisions

"Established confinement options are as applicable to field introductions of plants modified by molecular and cellular methods as they are for plants modified by classical genetic methods."

- Cited isolation requirements and other methods for production of genetically pure seed established by AOSCA

- Acknowledged that these "allow for acceptable levels of contamination".

1989 National Research Council Report Field Testing Genetically Engineered Plants: Framework for Decisions

Other proven routinely applied confinement methods include:

- Biological (sterility, reduced survival or fitness)
- Chemical (e.g. pesticides)
- Physical (e.g. fencing, caging, bagging of flowers)
- Geographical location, choice of environment
- Temporal (early termination, planting or flowering)
- Limitation of field plot size

1991 ABRAC Guidelines for Research

- Defined confinement, principles and practices
- Confinement measures should correspond to the level of safety concern
- Monitor to evaluate confinement measures

- Canola case study:
  - AOSCA isolation distance for foundation seed are appropriate to reduce cross-pollination to less than 0.05% for traits with low safety concern.
  - More or less stringent confinement methods are appropriate depending on safety of gene, the environment, and presence of sexually compatible plants.

1990- 1993 APHIS Sponsored Workshops on Safeguards for Field Testing of GE Crops

- Public meetings with domestic and international experts including breeders.
- Corn and wheat, tomato, rice, canola, potato, and sorghum

Focus was to:

- identify the potential for gene movement
- determine possible consequences of gene transfer and/or expression from these crops on agriculture and the environment
- recommend specific physical, temporal, or biological safeguards to prevent or mitigate such consequences, if appropriate

Introductions under 7 CFR 340: Permits vs. Notification

	Permit	Notification
Organisms Eligible	All	Plants, no noxious weeds
Traits	No specific restrictions	Certain risk-based restrictions; e.g. pharmaceuticals, industrials
Confinement measures	Applicant provides details, APHIS issues conditions	Applicant certifies compliance with Performance Stds.
Reviews: Field test Importation Movement State Concurrence	Up to 120 days 60 days 60 days Yes	30 days 30 days 10 days Yes
Site Inspections Field Data Reports	Yes Due after 6 mo.	Yes Due after 6 mo.

Guidance on Meeting the Performance Standards

- Eliminate viable vector agent with antibiotics
- Contained shipping and maintenance at facilities to prevent release of viable material – 7 CFR 340.8
- Prevent inadvertent mixing from planting
  - Maintain appropriate alley ways for equipment
  - Clean farm implements
- Maintain identity of transgenic material in use and devitalize when no longer in use.

Guidance on Meeting the Performance Standards

- Regulated article or its offspring can not persist in the environment.

Points to Consider:

- Termination prior to flowering, inhibit or remove flowers, use male sterility
- Reproductive confinement measures: bagging, wind breaks, border rows, temporal differences, isolation distances (e.g. AOSCA Stds for foundation seed production)
- Proximity to sexually compatible species, flowering cycles, extent of outcrossing and pollen dispersal
- Seed dispersal by biological or physical mechanisms

Guidance on Meeting the Performance Standards

- **Viable material is removed and volunteers are monitored and managed to prevent persistence.**

Points to Consider:

- **Harvest and destroy or contain propagative material**
- **Herbicide treatment of vegetative material**
- **Disc, cut, chip, mulch, or bury vegetative material for decomposition on site**
- **In subsequent growing season(s),**
  - **Consider land use restrictions / herbicide trt.**
  - **Monitor as long as seed could remain dormant under field conditions**
  - **Destroy volunteers prior to flowering**

Introductions of PMPs and PMIs Require an APHIS Permit

Information required for the permit described in:

- 7 CFR 340.4
- APHIS User's Guide
- FR Notices
- Letters to Applicants, Website

Nonregulated Status

- Not expected For most PMP/PMIs
- No Food or Feed Use - without devitalization and FDA or CVB clearances

Information Relative to Confinement Submitted for an APHIS Permit for Field Release of PMP/PMIs

- **Final and intermediate destinations**
- **Environment and conditions of the release**
- **Measures for physical and reproductive isolation from planting to harvest**
- **Site security, monitoring, and inspection**
- **Plans for termination, devitalization, disposal, and post-harvest monitoring and land use**

Sept. 2002 - FDA/USDA Draft Guidance to Industry:
Regarding Confinement for Commercial Production of PMPs

- **Procedures or genetic mechanisms to prevent PMP plants/seeds/products from entering the food/feed supply**
- **Tests to detect target gene and product**
- **IP system to track seeds from shipping to planting, and from harvest to extraction**
- **Harvesting procedures, including equipment identification and cleaning**
- **Appropriate disposal of wastes**
- **Federal government auditing of the system**

APHIS March 10, 2003 FR Notice
Current Supplemental Permit Conditions and Agency Actions for PMPs/PMIs

- Confinement measures and their implementation
  - Equipment use and cleaning
  - Storage facilities
  - SOPs
  - Fallow zones, land use restrictions, reproductive isolation,
  - Training programs
- APHIS will consider variances, case-by-case
- APHIS increased inspections, auditing to ensure compliance

Planters and Harvesters

- Dedicated to use in the permitted test site(s) for the duration of the test
- Notify APHIS if moved between test site(s)
- APHIS-approved cleaning procedures
- APHIS inspection before use other than permit use

All Other Field Equipment

- Cleaned according to APHIS approved protocols

Storage Facilities

- Dedicated facilities for the storage of equipment and regulated articles
  - locked or secure buildings, bins, or areas, posted as restricted to authorized personnel
- Cleaning per APHIS-approved protocols, and inspection prior to general use

Seed Cleaning and Drying

- APHIS-approved protocols to minimize seed loss/spillage

Fallow Zones and Land Use Restrictions

- Increased perimeter fallow zone to 50 ft. - allows movement of equipment without entering or commingling with adjacent food/feed crops
- Restricted production of food/feed crops for the following growing/volunteer monitoring season in cases where volunteer plants could be harvested with the following crop
- Does NOT exclude cover crops
- Criteria developed for variances, a few granted

Regulatory Compliance Mechanisms: Reporting Requirements

- Planting and harvesting notice
- Notice of equipment movement/cleaning
- 4-week post-planting report
- Field test reports due 6 months after field test
- Termination and interim reports may be required
- Post-season report on destruction of volunteers

Crop Specific Confinement Conditions

- FR Notice only addresses corn – WHY?
  - Most PMP/PMIs were using corn as platform
  - Larger acreage in field trials and production
  - Higher % outcrossing compared to other crops
  - Concern over food/feed adulteration
  - Phase out use of border rows
- Other crops are case-by-case, applicant driven
- Data must support confinement measure, with adequate margin for error
- APHIS encourages use of alternative plants

Field Tests of PMPs/PMIs

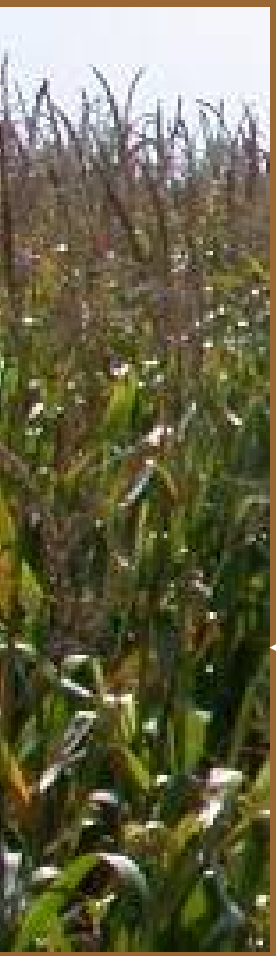
	2002	2003	2004
Acres	134	25	<44

2004 Platforms*	# Applicants
Corn	3
Barley	1
Rice	1
Safflower	1
Tobacco	1

•Excludes phytoremediation,TMV; rice and barley are for value - added proteins.

Corn Confinement Options

50 ft fallow zone with no food or feed crop



If Open Pollinated PMP Corn

1 mile (= 1.6 km) No Corn

If Controlled Pollination of PMP Corn

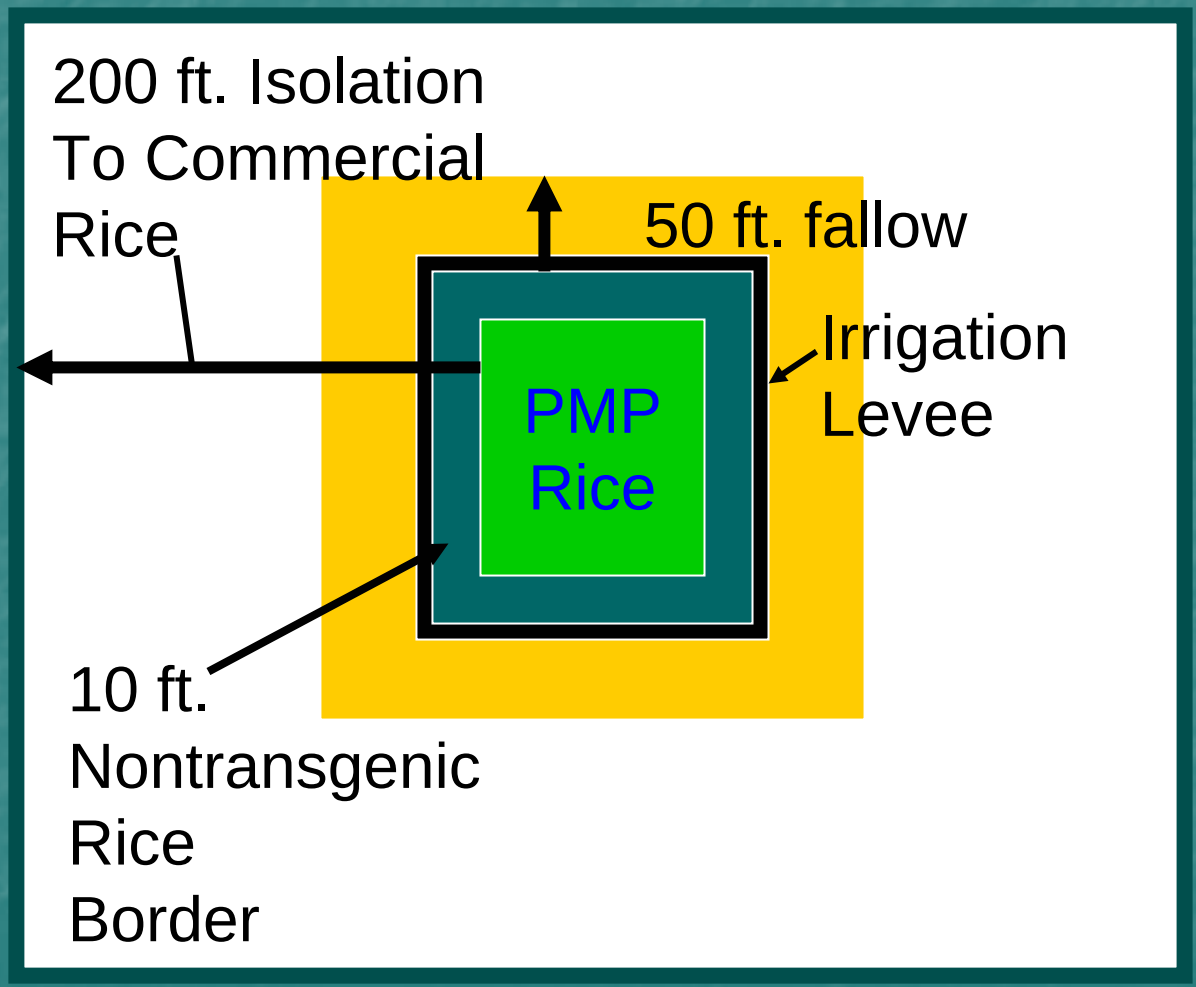
½ mile (= 0.8 km)
No Corn

½ - 1 mile
28 Day
Temporal Isolation

Rice
Confinement Considerations

- **Highly Self–pollinated**
  - **Only ~ 3.9% natural cross-pollination by wind, most within 2 meters (Virmani, 1994)**
  - **AOSCA, CCIA, foundation seed isolation is 10 ft.**
  - **IRRI uses 10 meter isolation – has seen no gene flow at 10 & 20 meters (Clegg et al., 1993)**
  - **Gene flow study in CA - no gene flow at even shorter distances**
- **Pollen loses viability in 5 minutes**
- **Transplanted or drill-seeded into flooded fields**
- **No seed dormancy**
- **Rice is not weedy, no weedy relatives at site**

Rice
Confinement Measures



Safflower Confinement Measures

- 50 ft. fallow border around the test
- 2 mile isolation from other safflower
  - Mostly self-pollinating- 4 wk. bloom stage
  - <10% outcrossing – pollinator mediated
  - No known sexually related species in the USA
- 2 year monitoring for volunteers within test area and fallow zone
  - safflower not recognized as having seed dormancy, but related species do
- No harvest of food/feed crops from monitoring area without APHIS permission.
- Dedicated planting and harvesting equipment
- Thorough cleaning of equipment used in the plant production and seed transport.

Future Challenges:
Evaluation of Genetic/Biological Confinement Methods

How can the value of these mechanisms be safely, accurately, and appropriately evaluated and applied for different crops.

Genetic and Genetic-Management of Gene Flow by:

Pollen

- Male sterility
- Chloroplast transformation
- Cleistogamy (and other effects to flowering)
- Apomixis

Seed sterility

Other

- Transgene linked with domestication trait(s)
- Induced expression
- Two-part systems

Daniell, H. (2002) *Nature Biotech.* **20**: 581
Gressel, J. (2002) in *Proceedings of the 4th Intl. Symposium on Biosafety*

Future Challenges: Effects of Scale and Use of Models to Evaluate and Compare Confinement Methods

What are the best models for extrapolating gene flow data generated:

- From small scale plots to large scale plots,
- From commercial crops to feral populations or wild relatives

Can/Do models account for:

- Combinations of confinement methods
- Size ratio of the transgenic pollen or seed source and the receiving population or border rows
- Their spatial distribution
- Distribution and abundance of pollinators and competing plants
- Topographic features that limit wind-assisted dispersal
- Seed loss or dispersal by equipment or other means

APHIS

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Rx

August 6, 2003 Interim Rule: "Introductions of Plants Genetically Engineered to Produce Industrial Compounds"

Plants engineered to produce industrial compounds include those plants that meet the following three criteria:

1) The plants are engineered to produce compounds that are new to the plant;

2) The new compound has not been commonly used in food or feed; and

3) The new compound is being expressed for non-food, non-feed industrial uses.

1990 Corn Safeguards Workshop

Conclusions:

- Isolation distances for seed certification (660 ft.) are adequate to minimize gene flow
- Temporal safeguards (altered planting date) could be used in combination with other methods
- Male sterility is useful, but may pose other risks
- No wild relatives in the U.S., but care should be taken in centers of origin
- Physical and spatial methods are appropriate for small scale tests, even multiple sites
- Safety issues should be resolved before larger scale releases

1990 Oilseed Crucifer Safeguards Workshop

Conclusions:

- **Hybridization and introgression into wild and weedy relatives in the US and elsewhere was a concern over the long term**
- ***B. napus* (canola) has lower rates of out-crossing than does *B. rapa* (rapeseed).**
- **Gene flow declines exponentially from the source, but with potential for low frequency long distance pollinator-mediated gene flow.**

	B. napus	B. rapa
46 meters	2.1 %	8.5 %
137 meters	1.1 %	5.8 %
366 meters	0.6 %	3.7 %

1990 Oilseed Crucifer Safeguards Workshop

Safeguarding suggestions:

- **Isolation distances (exclusion zones) for pedigreed seed:**
  - **400 meters for rapeseed; 200 meters for canola**
  - **Some suggested up to 4 km or 3 miles**
  - **Exclude wild relatives**

- **Border rows to attract pollinators to drop their load**
- **Male sterility, unless 100%, should not replace isolation**
- **Pollination cages useful for small plots**
- **Bird covers and cannons to prevent seed dispersal**
- **4 year monitoring period for volunteers**
  - **Rotate site and 100 ft. margin to another non-compatible crop and treat with herbicides**
  - **Methyl bromide treatment**

1992 International Consultation on Rice Biosafety

Conclusions:

- **Cultivated rice is highly self-fertilizing**
  - **~ 0.45 % outcrossing, effected by temperature and humidity**
- **Short pollen viability (about 5 minutes)**
- **Flowers open only 25-90 minutes, in a way that favors selfing**
- **Isolation distances:**
  - **10 meters (used by IRRI for breeding) may be appropriate for low risk situations**
  - **Recommended 20-40 meters - IRRI found no gene flow**

1992 International Consultation on Rice Biosafety

Conclusions:

- **Successful hybridization is limited to species with AA genomes, which in the U.S. includes only O. sativa and its weedy red rice variety**
- **Red rice has higher outcrossing rates, easy seed shattering, seed dormancy, and is later maturing.**
- **It is a concern only in the SE-U.S., but can be controlled by use of crop rotation, clean seed, and water seeding.**